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Crystallization and synchrotron X-ray diffraction studies of human interleukin-22

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Human interleukin-22, a novel member of the cytokine family, has been crystallized in hanging drops using the vapour-diffusion technique. Preliminary X-ray diffraction experiments using synchrotron radiation reveal that the protein crystallizes in space group $P2_12_12_1$, with unit-cell parameters a = 55.44, b = 61.62, c = 73.43 Å, and diffracts beyond 2.00 Å resolution.

1. Introduction

Interleukin-22 (IL-22), also known as interleukin-10-related T-cell-derived inducible factor (IL-TIF), a protein which shares 22% identity with interleukin-10 (IL-10), has been recently identified in interleukin-9 (IL-9) induced murine T cells (Dumoutier, Louahed *et al.*, 2000) and subsequently in human cells (Dumoutier, Van Roost, Colau *et al.*, 2000) as a novel member of the cytokine family. *In vitro* experiments showed that expression of IL-22 is rapidly induced by IL-9 in T cells, with maximal levels reached in 1 h, and that this induction does not require protein synthesis as the upregulation of the IL-22 mRNA is not blocked by cycloheximide (Dumoutier, Louahed *et al.*, 2000). This new protein, like other cytokines, has an approximate size of 20 kDa and an N-terminal hydrophobic signal peptide. Human and mouse IL-22 (hIL-22 and mIL-22) share 79% amino-acid sequence identity and both have 179 amino-acid residues.

Cytokines exert their actions by binding to specific cell-surface receptors, which leads to the activation of cytokine-specific signal transduction pathways. Two distinct receptor chains from the class II cytokine receptor family have been identified as taking part in the IL-22–receptor complex (Xie *et al.*, 2000; Kotenko *et al.*, 2001). These receptors are the CRF2-9 (IL22-R) and the CRF2-4 (IL-10R2 or IL-10Rβ), the latter also being a functional component of the IL-10 signalling complex (Kotenko *et al.*, 1997). This is the first scientific observation within the class II cytokine receptor family of a receptor being utilized as a component of multiple distinct cytokine signalling complexes.

It has been demonstrated *in vivo* that mIL-22 expression is rapidly increased in several mice organs after lipopolysaccharide (LPS) injection, indicating that the role of IL-22 may not be restricted to the immune system and that it is also involved in inflammatory responses (Dumoutier, Van Roost, Colau *et al.*, 2000). The latter is corroborated by the fact that IL-22 stimulation of HepG2 human hepatoma cells up-regulated the production of acute phase reactants such as serum amyloid A, α1-antichymotrypsin and haptoglobin (Dumoutier, Van Roost, Colau *et al.*, 2000). Moreover, there are data linking IL-22 to asthma and allergy owing to the probable involvement of IL-9 in these two pathologies (Temann *et al.*, 1998; McLane *et al.*, 1998; Levitt *et al.*, 1999). Other evidence for this linkage could be obtained at the DNA level. The hIL-22 gene is located on chromosome 12q (Dumoutier, Van Roost, Ameye *et al.*, 2000), where several loci potentially linked to asthma have been identified (Cookson, 2000).

In this paper, we describe the crystallization and results of preliminary X-ray diffraction studies of recombinant hIL-22 (rhIL-22) at 2.00 Å resolution.

2. Protein purification

Human IL-22 (corresponding to amino acids Gln29–Ile179; without the signal peptide) was cloned and expressed in *Escherichia coli* strain BL21 codon plus-(DE3)-RIL (Stratagene) as described in Dumoutier, Van Roost, Colau *et al.* (2000). Cells containing rhIL-22 were disrupted and inclusion bodies were collected by centrifugation. They were washed extensively first with 50 m*M* Tris–HCl, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 0.5%(*w*/*v*) deoxycholate (DOC) pH 8 and finally with the same buffer without detergent. Inclusion bodies were solubilized overnight in 8 *M* urea, 50 m*M* MES, 10 m*M* EDTA, 0.1 m*M* DTT pH 5.5. The rhIL-22 protein was refolded by direct dilution of the solubilized inclusion bodies in the following folding mixture: 100 µg ml^{-1} rhIL-22, 100 m*M* Tris–HCl, 2 m*M* EDTA, 0.5 *M* L-arginine, 1 m*M* reduced glutathione, 0.1 m*M* oxidized glutathione pH 8. The solution was incubated for 72 h. The folding

mixture was then concentrated by ultrafiltration in an Amicon chamber with a YM3 membrane before purification on a Superdex75 (Amersham Pharmacia Biotech) gel-filtration column. The protein was eluted with 25 m*M* MES, 150 m*M* NaCl pH 5.4. Recombinant human IL-22 peak fractions were concentrated to 5 mg ml^{-1} with a YM3 Amicon membrane and desalted using a Hi-Prep 26/10 column (Amersham Pharmacia) with elution buffer containing 10 m*M* MES pH 5.4. The protein was concentrated again to 5 mg ml^{-1} and lyophilized in 1 mg fractions.

3. Crystallization and data collection

Initial crystallization conditions were screened by the sparse-matrix method using the macromolecular crystallization reagent kits Crystal Screen I and II (Hampton Research) at 291 K. Small crystals were found in conditions number 18, 26 and 29 of the Crystal Screen I kit. In each trial, a hanging drop of 1 µl protein solution (10 mg ml^{-1} in 20 m*M* MES buffer pH 5.4) was mixed with 1 µl precipitant solution and equilibrated against a reservoir containing 500 µl precipitant solution. Several attempts to improve crystal quality were performed, including pH and precipitant concentration refinement, detergent addition and macroseeding. Well diffracting crystals were finally obtained using a precipitant solution consisting of 0.9 *M* sodium tartrate, Triton X-100 detergent and 0.1 *M* HEPES pH 7.5. Two synchrotron X-ray diffraction data sets, one native and one derivative, have been collected for this study. Both data sets were collected using a 345 mm MAR Research image-plate detector at the Brazilian National Synchrotron Light Laboratory protein crystallography beamline (Polikarpov, Perles *et al.*, 1997; Polikarpov, Oliva *et al.*, 1997) by the oscillation method at 100 K. The native crystal was immersed for 30 s in a cryocooling solution (precipitant solution with 15% ethylene glycol), mounted in a rayon loop and flash-cooled to 100 K in a cold nitrogen stream. An iodine derivative was prepared following the quick cryosoaking derivatization procedure (Dauter *et al.*, 2000; Nagem *et al.*, 2001). A single crystal was immersed for 3 min in a cryocooling solution (the same as used for the native crystal) additionally containing 0.125 *M* sodium iodide and then frozen in a rayon loop in a cold nitrogen-gas stream (100 K). The synchrotron radiation wavelength was set to 1.54 Å to optimize the X-ray flux and to increase the anomalous signal for the iodine derivative. The initial images of each data set were subjected to the autoindexing routine of *DENZO* (Otwinowski & Minor, 1997), which suggested a primitive orthorhombic cell to be the best solution. Following an optimum strategy of data collection suggested by the program *marHKL*, totals of 103 and 248° of native and iodine-derivative data were collected, respectively. The images were processed and scaled with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Native and iodine-derivative crystal data and data-collection statistics.

Values for the highest resolution shell are shown in parentheses.

	Native	Iodine derivative
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 55.44, b = 61.62, c = 73.47	a = 56.05, b = 61.78, c = 73.63
Resolution (Å)	21.7–2.00 (2.05–2.00)	21.8–1.92 (1.96–1.92)
No. of reflections	61846	182876
No. of unique reflections†	16382	37777
$\langle I/\sigma(I)\rangle$	14.5 (3.8)	13.4 (3.1)
Multiplicity	3.8 (3.4)	4.8 (4.3)
Completeness (%)	92.7 (82.3)	99.9 (99.7)
$R_{\rm merge}$‡ (%)	8.2 (35.0)	11.7 (43.9)
$R_{\rm fac}$§ (%)		21.7
Data collected (°)	103	248
Cryoprotectant solution	Mother liquor, 15% ethylene glycol	Mother liquor, 15% ethylene glycol, 0.125 *M* NaI
Soaking time (s)	30	180

† Multiplicity of derivative and native data sets calculated with Friedel-related reflections treated separately and as equivalent, respectively. ‡ $R_{\rm merge} = \sum_{hkl}|I_{hkl} - \langle I_{hkl}\rangle|/\sum_{hkl} I_{hkl}$. § $R_{\rm fac} = \sum_{hkl}|F_{PH} - F_P|/\sum_{hkl} F_P$.

The iodine derivative, contrary to most derivatives prepared by traditional soaks, did not suffer a considerable loss of diffraction power, modification of unit-cell parameters or degradation during its preparation and has a great chance of being isomorphous (Table 1).

A search for additional heavy-atom derivatives and phase calculations using the SIRAS method are currently under way.

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